

Review

Membrane binding domains

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Abstract

Eukaryotic signaling and trafficking proteins are rich in modular domains that bind cell membranes. These binding events are tightly regulated in space and time. The structural, biochemical, and biophysical mechanisms for targeting have been worked out for many families of membrane binding domains. This review takes a comparative view of seven major classes of membrane binding domains, the C1, C2, PH, FYVE, PX, ENTH, and BAR domains. These domains use a combination of specific headgroup interactions, hydrophobic membrane penetration, electrostatic surface interactions, and shape complementarity to bind to specific subcellular membranes.

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1. Introduction

Eukaryotic cells are defined by their membrane-delimited organelles. Signals are transduced across membranes by machinery that must be at the membrane. Organelle biogenesis and identity depends on the protein and lipid composition of organellar membranes. Most eukaryotic proteins are modular in structure and contain one or more conserved domains. Given the diversity of membrane structures in eukaryotic cells and the complexity of domain structures in eukaryotic proteins, it makes sense that there so are many conserved membrane and lipid binding domains in the eukaryotic proteome. These domains are most abundant in proteins of signal transduction and protein and membrane trafficking, since these are processes that are carried out at cell membranes. My aim in this chapter is to provide an overview of these domains. I will draw selectively on the literature for a subset of the best understood domains in order to illustrate principles, while the reader is referred to other chapters in this volume for comprehensive reviews of individual domains.

2. Archetypal lipid binding domains

The history of membrane-binding domain research began with Nishizuka's discovery of protein kinase C (PKC) in the late

1970's. This discovery was followed by the cloning of several PKC isozymes and the observation of two regions of conserved sequence in the regulatory part of the enzyme. One of these regions, denoted C₁ (for conserved region 1) at the time, was found to be responsible for phorbol ester binding [1]. We now know that this conserved region corresponds to two structural C1 domains that can bind to phorbol esters, diacylglycerol (DAG), and membranes [2,3]. The second region, C₂, is responsible for Ca²⁺ dependence of conventional PKC activation [1]. This region corresponds directly to the C2 structural domain that binds to Ca²⁺ in isolation, as shown first for another C2 domain protein, synaptotagmin I [4]. Just as the discovery of PKC launched the field of lipid second messenger signaling, the discovery of the C1 and C2 domains launched the field of membrane binding domains in signal transduction. It is fitting that the third major membrane binding domain to be described, the pleckstrin homology (PH) domain, also owes its discovery in part to the PKC field. Pleckstrin was characterized as the major PKC substrate in platelets in the late 1980s, and the PH domain was discovered and named as a conserved region present in pleckstrin and other proteins implicated in signaling [5,6]. The PH domain of phospholipase C- δ was the first to be established as a high-affinity membrane binding domain [7–10], in this case one specific for the lipid phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P₂). The year 1995 saw the first structural studies of high-affinity complexes of the C1 [11], C2 [12], and PH

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domains [13] with their ligands phorbol ester, Ca^{2+} , and inositol (1,4,5)-trisphosphate (the headgroup of $\text{PI}(4,5)\text{P}_2$). These and other studies on C1, C2, and PH domains in the years 1989–1995 spawned the membrane binding domain field and sketched its broad biochemical and biophysical principles.

The past decade has seen the expansion of membrane-binding domain studies *in vivo* and on a genomic scale, a continuing increase in the sophistication of biochemical and biophysical studies, and the introduction of many new members to this class of domains. 1998 was a seminal year in the membrane-binding domain field in that the use of green fluorescent protein (GFP) fusions was introduced into the field and first used to probe the dynamics of diacylglycerol [14], Ca^{2+} [14], and $\text{PI}(4,5)\text{P}_2$ [15,16] in real time in living cells (see Balla chapter). A host of new membrane binding domains have been identified, including FERM (Four point one-ezrin-radixin-moesin), PX (phox), FYVE (Fab1/YOTB/Vac1/EEA1), Tubby, BAR (Bin/Amphiphysin/Rvs), and ENTH/ANTH (Epsin/AP180 N-terminal homology) [17–21]. The functions of the PH and PX domain families have been analyzed across the yeast genome, putting these domains into genomic perspective [22].

The major dilemma for structural biologists and biophysicists working in this field is that the structures of domains embedded in membranes are not approachable by X-ray crystallography. The smallest domains are approachable by solution NMR when embedded in short-chain lipid micelles, although not in bilayer membranes. This has been a major handicap to the advance of the field compared to areas of structural biology where functional complexes can be visualized at high resolution. A number of creative approaches are now being applied to fill in the gaps. The application of site-specific labeling for fluorescence resonance energy transfer (FRET) and electron paramagnetic resonance (EPR; [23] and see Cafiso chapter) was pioneered in studies of C2 domain:membrane interactions. These techniques now appear to be mature enough to be applied to other membrane-binding domains and perhaps to larger membrane-associated assemblies nucleated by these domains. Computational analysis of electrostatic interactions is rapidly becoming a standard part of membrane:domain interaction studies (see Murray chapter). All-atom molecular dynamics of membrane binding domains embedded in phospholipid bilayers is computationally demanding, but with current computational capabilities is now beginning to contribute to the field [24]. The application of X-ray reflectivity studies to membrane-bound domains, again using the C2 domain as a test case [25], is in its early stages but appears to be a very promising complement to these other approaches.

2.1. C1 domains

The C1 zinc finger domains of the conventional and novel (i.e. α , βI , βII , γ , δ , ϵ , θ , η , and μ , but not λ/ι or ζ) PKCs, chimerins, RasGRPs and Unc-13 are known as the “typical” C1 domains, and are specific receptors for DAG and phorbol esters (see [26] and Kazanietz chapter). These C1 domains bind stereospecifically to phorbol esters, and presumably DAG, through a narrow polar groove between two pulled-apart β -

strands [11]. The groove is surrounded by an exposed ridge of hydrophobic residues [11] that penetrate into the hydrocarbon core of the membrane [27,28] (Fig. 1). The Zn-finger structure of the C1 domain is thought to stabilize its fold in both its inactive, soluble state and its active, deeply membrane-embedded state. A belt of basic residues covers the middle portion of the domain, adjacent to the hydrophobic ridge at tip. These residues are thought to bind to the acidic headgroups of phosphatidylserine [29] at the surface of the membrane. C1 domains bind to phorbol esters embedded in acidic phospholipid membranes or micelles with $\sim 10^4$ -fold higher affinity than to short-chain phorbol esters in free solution [30]. The interplay between the polar groove, hydrophobic ridge, and basic belt of the C1 domain is essential for high affinity binding to membrane-embedded phorbol esters and DAG.

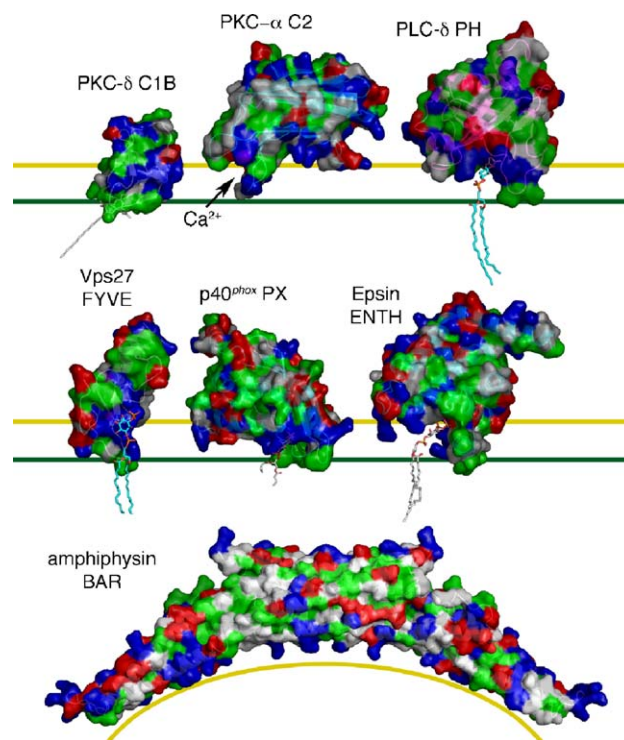


Fig. 1. Molecular surface models of membrane binding domains. Surfaces are rendered with hydrophobic residues in green, basic in blue, acidic in red, and uncharged polar in white. The yellow line marks the top of the polar interface region of the bilayer, and the green line marks the top of the hydrocarbon core of the bilayer. The C1B domain of PKC- δ bound to phorbol 13-acetate (1PTR) [11] was docked to the membrane on the basis of structural modeling and solution NMR of the related PKC- γ C1B domain [27]. The C2 domain of PKC- α (1DSY) [39] was docked on the basis of FRET and EPR analysis [78]. The PH domain of PLC- δ (1MAI) [13] was docked on the basis of structural modeling. The FYVE domain of Vps27 (1VFY) [56], with $\text{PI}(3)\text{P}$ docked on the basis of its binding site in EEA1 [58], was docked to the membrane based on structural and electrostatic modeling [74]. The PX domain of p40^{phox} bound to $\text{PI}(3)\text{P}$ (1H6H) [62] was docked on the basis of structural modeling and demonstrated membrane penetration [77]. The ENTH domain of epsin (1HOA) [63] bound to $\text{PI}(4,5)\text{P}_2$ was docked on the basis of structural modeling and demonstrated membrane penetration [79]. The amphiphysin BAR domain (1URU) [68] was docked to a curved membrane surface based on shape complementarity. Phospholipid and phorbol ester tail moieties were modeled arbitrarily except for the PX domain, where the crystallized conformation is shown.

The C1 domain provided an early and vivid example of how the three major structural features of membrane binding domains work together: (1) a polar pocket or groove that stereospecifically recognizes a specific ligand; (2) a hydrophobic protrusion that penetrates into the hydrocarbon core of the membrane; and (3) clusters of basic residues that bind to acidic phospholipid headgroups (Fig. 2). Of course, not all of these features are present in all membrane-binding domains. These features and the mechanisms associated with them are used to widely varying degrees in different families of membrane binding domains, and even among different instances of a given type of domain.

The earliest studies of PKC focused on its activation by lipids and Ca^{2+} , as opposed to subcellular targeting. The membrane engagement of the C1 domain was long known to allosterically activate the kinase, and the general outlines of a biochemical activation mechanism are known [31,32]. The detailed structural mechanisms for enzyme activation by C1 and other membrane binding domains have lagged far behind analysis of targeting mechanisms, however. The mechanism for C1 domain-based activation has been worked out for one case, the RacGAP protein $\beta 2$ -chimaerin [33]. In the soluble and enzymatically inactive conformation, the $\beta 2$ -chimaerin C1 domain cooperates with an N-terminal inhibitory region to sterically block Rac binding. In this conformation, the hydrophobic ridge of the C1 engages in intra-protein interactions with other domains of $\beta 2$ -chimaerin. Membrane binding requires that these inhibitory intraprotein interactions be broken up, allowing Rac access to the catalytic RacGAP domain. The structural mechanism for PKC activation is likely to be similar, but elucidating the details remains an important challenge for the field.

2.2. C2 domains

The C2 domain is best known as the Ca^{2+} sensor in the conventional PKCs (α , βI , βII , γ) and synaptotagmin I, although many C2 domains in other proteins do not bind Ca^{2+} at all (see [34] and Cho & Stahelin chapter). C2 domains are β -sandwiches whose tip binds to between 2 and 3 Ca^{2+} ions via three loops known as Ca^{2+} binding regions (CBRs) [12,35,36]. For most Ca^{2+} -binding C2 domains, the binding of the Ca^{2+} ions potentiates binding to acidic phospholipid membranes, with typically low specificity for one acidic lipid over another [37]. The C2 domain of cytosolic phospholipase A_2 (cPLA $_2$),

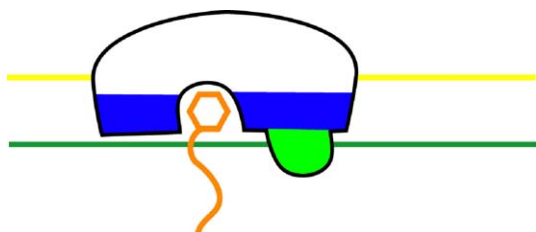


Fig. 2. Conceptual model for membrane binding domain interactions. An idealized membrane binding domain is shown in the same color scheme used in Fig. 1.

however, binds preferentially to neutral membranes in its Ca^{2+} -bound state [37]. The C2 domains of synaptotagmin I, PKC α , and PKC β have basic residues surrounding the CBRs [12,38,39], while the cPLA $_2$ C2 domain has a unique amphipathic helix whose hydrophobic face penetrates the membrane in the Ca^{2+} -bound state [40,41]. A number of mechanisms have been proposed to explain how Ca^{2+} can promote binding to acidic membranes in some cases, and neutral membranes in others, including direct Ca^{2+} -phospholipid bridging, Ca^{2+} -induced conformational changes, and electrostatic control (reviewed in [17]). A computational electrostatic model has been developed that appears to explain the observed specificity differences through changes both in interaction energies and in the penalty for desolvating charges near the membrane [42]. Considerable effort has gone into defining the geometry of C2 domain interactions with membranes, making this field a test bed for applying FRET, EPR, X-ray reflectivity, and computational methods ([23]; Cafiso chapter).

2.3. PH domains

The PH domain family is the largest and most functionally diverse in lipid signaling (see [20,43] and Lambright chapter). The PH domain fold also crops up as a substructure within the FERM domain [44]. One of the first functions reported for a PH domain was for that of the kinase GRK2 (formerly $\beta\text{ARK}1$), which binds to the $\text{G}\beta\gamma$ complex through its PH domain [45]. NMR studies of the pleckstrin PH domain highlighted its ability to bind PI(4,5)P $_2$ in micelles with moderate affinity and specificity [46]. A subset of PH domains, such as those from PLC- δ , GRP1, BTK, and PDK1, bind phosphoinositides with high affinity and specificity, and have perhaps received the most attention [19,20]. It is ironic that the high-affinity group is a small fraction of the total, and that the great majority of PH domains either bind phosphoinositides with low to moderate affinity and specificity, or do not bind phosphoinositides at all [47].

The specificity determinants of these high affinity PH domains reside in basic residues in loops connecting the β -strands, which surround a deep pocket (for examples see [48,49]). The surface corresponding to this pocket is less defined in the lower specificity PH domains, such as pleckstrin and spectrin [46,50,51]. The protein:protein interaction surface on the GRK2 PH domain is distinct from its membrane-binding surface [52], such that both interactions can occur simultaneously and synergistically. Unlike C1 domains, PH domains do not typically have large hydrophobic protrusions. This explains why PH domains such as that of PLC- δ bind to soluble lipid headgroups and membranes with comparable affinities [9], in contrast to the 10^4 -fold difference for C1 domains.

3. Membrane binding domains in subcellular trafficking

The roots of the membrane binding domain field are in classical receptor-mediated signal transduction at the plasma membrane. Much of the growth in the field has centered on the

role of membrane binding domains in vesicular trafficking, however. Some of the highlights in this area have been the discovery and structural characterization of FYVE [53–58] and PX [59–62] domains as receptors for the endosomal lipid phosphatidylinositol 3-phosphate; the characterization of high-affinity PI(4,5)P₂ binding sites on endocytic proteins and complexes such as epsin [63] and AP180 [64–66], and the role of BAR domains and amphipathic α -helices in sensing and inducing membrane curvature [63,67–70].

3.1. FYVE domains

FYVE domains mediate the endosomal targeting of diverse proteins by binding with high affinity and specificity to PI(3)P ([71] and Kutateladze chapter). FYVE domains are Zn fingers that are distant structural cousins of C1 domains [56]. The FYVE domain has a shallow basic pocket that specifically recognizes PI(3)P, additional basic residues for non-specific interactions with acidic phospholipid headgroups, and a hydrophobic protrusion that penetrates into the membrane [56–58]. The hydrophobic “turret loop” is located at essentially the same position as the membrane penetrating ridge in the C1 domain, although it is less extensive. As for the C1 domain, binding to PI(3)P and bulk phospholipid is synergistic [72] and involves membrane penetration [73]. The docking of FYVE domains onto short-chain lipid micelles has been extensively studied by NMR (Kutateladze chapter), since this domain is only ~60 amino acids and thus amenable to solution NMR approaches even with the added mass of a small lipid micelle. The role of electrostatics in FYVE domain:membrane binding has also been extensively probed by computational analysis ([74] and Murray chapter). The charge distribution on the surface of the FYVE domain appears to control the angle at which it docks onto membranes. While most FYVE domains bind specifically to PI(3)P, their membrane docking behavior seems to vary considerably. Fig. 1 shows the perpendicular orientation of the Vps27 FYVE domain. In contrast, the FYVE domain of EEA1 is tilted by about 50° to the perpendicular when it binds membranes [58,74,75]. Despite differences in docking angle, all FYVE domains analyzed use their turret loops to penetrate the membrane.

3.2. PX domains

Like FYVE domains, the PX domain family is specialized for endosomal targeting and phosphoinositide binding (Hong chapter). Yeast PX domains all bind PI(3)P [76], but mammalian PX domains have a more diverse repertoire of ligands. The PX domain is about twice the size of the FYVE domain and has a completely different fold, showing how convergent evolution has led to two potent but completely different mechanisms for binding PI(3)P [62]. PX domains have a deep pocket for specific binding to phosphoinositides, a basic surface surrounding it, and a significant hydrophobic protrusion near the pocket that penetrates membranes [77].

3.3. ENTH domains

ENTH domains are a motif of endocytic proteins that have a stable octahelical core and a conformationally dynamic N-terminal amphipathic helix (De Camilli & Itoh chapter). The core and the N-terminal helix fold together to bind to PI(4,5)P₂ [63]. The PI(4,5)P₂-bound conformation of the epsin ENTH domain penetrates deeply into membranes using the exposed hydrophobic face of the amphipathic helix [63]. This membrane penetration is thought to promote the positive curvature of nascent vesicles in endocytosis. Like FYVE domains, the ENTH contains both a specific phosphoinositide headgroup-binding pocket composed of basic residues and a hydrophobic membrane-penetrating protrusion. The distinctive feature of the ENTH domain compared to the FYVE domain and others is that the hydrophobic protrusion is conformationally labile and only becomes structured upon phosphoinositide binding.

3.4. BAR domains

BAR domains are helical dimers that do not recognize specific membrane lipids, but rather sense the curvature of a membrane (De Camilli & Itoh chapter and [69]). BAR dimers are shaped like bananas and bind curved vesicular membranes with their concave faces [68,69]. Portions of the concave faces are basic such that they can bind the surface of acidic phospholipid membranes. Thus, the BAR domain illustrates the use of basic surfaces for non-specific acidic membrane binding, as seen in many other domains. The BAR domains are specialized such that these surfaces complement those of curved tubules, the necks of budding vesicles, or other highly curved membranes [68]. While BAR domains lack specific lipid recognition elements, they often occur in conjunction with PX or other domains to provide targeting specificity. Isolated BAR domains appear to be curvature sensors, rather than inducers, but BAR domains coupled to membrane-penetrating amphipathic helices are potent inducers of curvature.

4. Structural principles for membrane binding domains

The comparative analysis of different membrane binding domains reveals several persistent themes. The C1, PH, FYVE, PX, and ENTH domains are examples of domains that contain a pocket for the stereospecific recognition of a unique lipid ligand. These domains also recognize the soluble headgroups of their lipid ligands. In some cases, where additional contacts with the membrane are modest, as for the PLC- δ PH domain, the soluble headgroup binds with comparable affinity to the membrane-bound lipid. In other cases, the presence of the surrounding membrane is required to make additional contacts. The typical C1 domains, the FYVE and PX domains, and the epsin ENTH domain are examples of domains with hydrophobic protrusions that penetrate substantially into the hydrocarbon core of the membrane. Membrane penetration can provide very large enhancements to the affinity of domain:membrane interaction, as highlighted by the 10⁴-fold preference for membrane-embedded vs. short-chain phorbol esters by the

PKC- δ C1B domain. Membrane penetration can modulate membrane structure in biologically important ways, as first shown for the epsin ENTH domain in endocytic pit formation. The PKC- α C2 domain and the amphiphysin BAR domain are examples of membrane binding domains that rely primarily on interactions with the electrostatic field created by multiple acidic phospholipid headgroups. For the C2 domain, Ca^{2+} appears to function as a switch to regulate binding to planar membranes, whereas the BAR domain is shaped such that it can only bind to highly curved membranes.

Since the dawn of the membrane binding domain field in 1989, there has been enormous progress in understanding the biological, biochemical, and biophysical mechanisms of these domains. The contributors to this volume and many other colleagues deserve congratulations for having taken the field so far. While most of the broad principles appear to be in hand, nature has a way of surprising us just when we think we have it all figured out. After all, it has only been 2 years since the explanation for curvature sensing by BAR domains was worked out. In my view, the major frontier in the field now is to integrate the wealth of information we already have on individual membrane binding domains. We need to understand how membrane binding domains function in the context of large multiprotein assemblies on membranes, and how they work in the context of the large multidomain proteins that do the work in eukaryotic signaling and trafficking.

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